guyanensis. No known viral contaminants, such as LRV1-4, were detected in the promastigotes. A clonal line, WR2334 Clone D2 (WR2334-D2), was established through serial endpoint dilution and microscopic visualization of a single promastigote. This source seed stock of this clone was then expanded in culture, aliquoted and cryopreserved in liquid nitrogen. Sterility and morphology were determined by conventional methods.

[49] A master cell bank for the *L. guyanensis* (WR2334-Clone D2) Leishmania Skin Test, LSTA-Lg (BPR-377-00, Lot No. 0705) was manufactured under defined cGMP conditions from cryopreserved aliquots of WR2334-Clone D2, by culturing and expansion to roller bottles in appropriate medium. The cultured promastigotes were harvested by centrifugation, washed, suspended in cryopreservation medium, aliquoted, and stored in liquid nitrogen. Sterility and morphology were determined by conventional methods.

[50] A working cell bank for the *L. guyanensis* (WR2334-Clone D2) Leishmania Skin Test, LSTA-Lg (BPR-377-00, Lot No. 0705) was manufactured under defined cGMP conditions from cryopreserved aliquots of WR2334-Clone D2 Master Cell Bank, by culturing and expansion to roller bottles in appropriate medium. The cultured promastigotes were harvested by centrifugation, washed, suspended in cryopreservation medium, aliquoted, and stored in liquid nitrogen. Sterility and morphology were determined by conventional methods.

[51] A bulk lot for *L. guyanensis* (WR2334-Clone D2) Leishmania Skin Test LSTA-Lg (BPR-383-00, Lot No. 0707) was produced. Specifically, bulk promastigotes were cultured in Schneider's Drosophila Medium (SDM), supplemented with 20% fetal bovine serum (heat inactivated). The inoculum was initiated in flasks and when the culture set point was reached, it was transferred to roller bottles and expanded. Promastigotes were harvested by centrifugation when a sufficient quantity was been obtained. The culture was maintained in healthy, log-growth phase throughout the production run, including the time of harvest. Harvested promastigotes were centrifuged and washed 5 times. The pellets were reconstituted in sterile saline USP and manually filled in 5.0 mls aliquots at 109 promastigotes/ml. The vials were labeled, stored and frozen at –80 °C for subsequent production and purification steps. Sterility and morphology were determined by conventional methods.

[52] The bulk lot for *L. guyanensis* (WR2334-Clone D2) Leishmania Skin Test LSTA-Lg (BPR-389-00, Lot No. 0716) was purified. Specifically, cryopreserved vials were

thawed and processed through a microfluidizer (MFL#1). The material was centrifuged at 3,100 rpm for 30 minutes and the supernatant was saved. The pellet was resuspended and microfluidized again (MFL#2). The material was centrifuged at 12,200 rpm for 30 minutes and the two supernatants combined. The pooled supernatants were placed in a 0.22 micron sterile filter unit (500 ml) and filtered. The filtrate was then heat-treated at 90 °C for 15-20 minutes to inactivate parasite proteases. The final filtered and heat-treated bulk was formulated and the protein concentration adjusted to about 0.35 mg per ml. The lot was bottled and stored at 4 °C. The bulk material was tested for the following characteristics: Visual Inspection: Color, Appearance; Protein Content: micro-BCA (prior to phenol addition); Purity/Identity: SDS-PAGE, HPLC; and Quality: Sterility; Rabbit Pyrogen, Endotoxin (LAL), pH.

[53] The final product of *L. guyanensis* (WR2334-Clone D2) Leishmania Skin Test LSTA-Lg (BPR-390-00, Lot No. 0717) was placed in vials. Specifically, the formulated, bulk LSTA-Lg was aseptically filled in 1.0 ml aliquots into 10 ml Type I glass vials, closed with 20 mm rubber closures and stored at 4°C. The samples were tested for the following characteristics: Visual Inspection: Color, Appearance, Homogeneity, and Viscosity; Purity/Identity: SDS-PAGE, HPLC; Quality: Sterility, Rabbit Pyrogenicity, General Safety, Endotoxin (LAL); pH; Potency: In vivo Immunogenicity (Guinea pig DTH); and Stability: Time Zero, 3, 6, 12 months, 1, 2 and 3 years at 4, 37, and –80°C

Example 2

Production of Heat-Treated Leishmania Skin Test Injectable

[54] Bulk lots of *L. mexicana* promastigotes in dry ice were pooled by thawing the bulk lots in a water bath at 56 ± 2 °C. Immediately upon thawing, the bulk lots were placed at 4 ± 2 °C to cool. After cooling, the contents of the vial were transferred to a pre-tared 250 ml sterile centrifuge bottle on ice using a sterile 5 ml pipet. Two 0.5 ml samples of the suspended cells were pipetted and placed in a 1.5 ml sterile Nunc cryovial (Fisher Scientific, Pittsburgh, PA) and stored at -80 ± 10 °C.

[55] A microfluidizer, Model #M-110S was washed and autoclaved per the manufacturer's directions. The regulator was set and wet ice was put into the cooling jacket of the microfluidizer. The pump was primed by placing the inlet tubing into 500 ml of 0.001% Tween 80 and 0.9% saline and then opening the air valve. With the regulator at 100 ± 5 psi, the inlet tubing was inserted into the promastigotes suspension

and run through the microfluidizer. Because the cracking pressure fluctuates, an average reading was taken. Cracked cells were collected into the reservoir containing the uncracked cells and cracking continued for 10 ± 1 minutes. The pre- and post-cracking temperatures were recorded. The 250 ml tube containing the lysed promastigotes was capped and stored at 4 ± 2 °C.

[56] The cracked cells were dispensed into a sterile 250 ml centrifuge bottle and centrifuged at 3,100 ± 200 rpm (1566 x g) in a Sorvall GSA rotor within a RC-5 Sorvall centrifuge at a time setting of 30 ± 1 minutes and at a temperature of 4 ± 2 °C. The bottle was removed and placed in a Class II Biohazard cabinet. The supernatant was poured of into a second sterilized 350 ml centrifuge tube and stored at 4 ± 2 °C. The pellet was then suspended with 20 ml of Buffer B, comprising 0.001% Tween 80 diluted with 0.9% saline, and vortexed with vortex mixer. Ice was added into the cooling jacket of the microfluidizer as needed. When the regulator was at 100 ± 5 psi, the inlet tubing was inserted into the suspension and run through the microfluidizer in a continual fashion for 10 minutes. Cracked cells were collected into a 250 ml centrifuge bottle containing the lysed promastigotes and placed on ice. The pre- and post-cracking temperatures were recorded. Then two 0.5 ml of the cracked cell samples were taken and stored at -80 ± 10 °C. On ice the cracked cells and the supernatant were combined and mixed by swirling in the container.

[57] To avoid any possibility of breakdown, the post-cracked cells were immediately centrifuged at 12,200 ± 200 rpm (23,435 – 25,062 x g) in a Sorval GSA rotor within a RC-5 Sorvall centrifuge for 30 ± 1 minutes at 4 ± 2 °C. Then the bottle was removed and placed in BSC, the supernatant was poured off into a sterile 250 ml graduated cylinder and the pellet in the 250 ml bottle was retained.

[58] A biosafety cabinet was sterilized as well as other items placed in the cabinet with 70% alcohol. A UV lamp was kept on in the cabinet for 15 minutes prior to use. The bulk lysate and centrifuged promastigotes were filtered. The volume of centrifuged promastigotes solution was estimated and the in process purified bulk lysate was aseptically filtered using a 500 ml 0.22 μm Millipore filtration unit (Fisher Scientific, Pittsburgh, PA). The filtered bulk was aseptically transferred into a preweighed, sterile bottle. The bottle of the bulk was reweighed and the bulk weight was determined. Two 0.5 ml aliquots were aseptically taken and retained at -80 ± 10 °C. One 1.0 ml sample